IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Andrew Robert Marks et al.	Confirmation No.: 7653
Application No.: 10/809,089	Patent No.: 7,718,644 B2
Filing Date: March 25, 2004	Patent Date: May 18, 2010
For: ANTI-ARRHYTHMIC AND HEART FAILURE DRUGS THAT TARGET THE LEAK IN THE RYANODINE RECEPTOR (RYR2) AND USES THEREOF	Attorney Docket No.: 86005-59600

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR § 1.323

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

It is requested that a Certificate of Correction be issued in connection with the above-identified patent correcting the errors listed on the accompanying Form PTO-1050. The corrections requested are as follows.

In column 14, please delete the text appearing in column 14, line 59, through column 15, line 42, and insert this deleted text in column 16, after line 34 and before the heading "DETAILED DESCRIPTION OF THE INVENTION". The description of the figures will then appear in the correct order.

In column 76, line 26 (claim 20, line 5), change "1 μM" to -- 1.0 μM --. Support for this change appears in application claim 60.

This request is being made pursuant to 37 CFR § 1.323 to correct errors of a clerical or typographical nature and do not involve changes that would constitute new matter or require reexamination. A fee of $100 is believed to be due for this request. Please charge the required fees to Winston & Strawn LLP Deposit Account No. 50-1814.

Please issue a Certificate of Correction in due course.

Respectfully submitted,

Date: November 16, 2010

Allan A. Fanucci, Reg. No. 30,256

WINSTON & STRAWN LLP
Customer No. 28765
212-294-3311

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,718,644 B2

APPLICATION NO. : 10/809,089

DATED: : May 18, 2010

INVENTOR(S) : Marks et al.

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Columns 14-15:
Delete the text beginning at line 59 through column 15, line 42, and insert this deleted text in column 16, after line 34 and before the heading "DETAILED DESCRIPTION OF THE INVENTION".

Column 76:
Line 26 (claim 20, line 5), change "1 μM" to -- 1.0 μM --.

WINSTON & STRAWN LLP
Patent Department
1700 K Street, N.W.
Washington, D.C. 20006-3817

FIG. **4** shows that JTV-519 improves cardiac contractility in a rat model of heart failure. (A) Myocardial diastolic cross-sectional area was determined at mid-papillary levels using echocardiography, before and after a 4-week treatment with JTV-519 or vehicle (control). The relative increase in diastolic dysfunction was inhibited by JTV-519. (B) Although systolic function deteriorated in non-treated animals, JTV-519 significantly increased systolic function in post-myocardial-infarction (post-MI) heart-failure rats.

FIG. **5** demonstrates that JTV-519 increases calstabin2 (FKBP12.6) affinity to RyR2 in heart-failure rats. Equivalent amounts of RyR2 were immunoprecipitated using anti-RyR2 antibody (A). Representative immunoblots (A) and bar graphs (B) show the amount of PKA phosphorylation of RyR2 at Ser2809 (B, left) and the amount of calstabin2 (FKBP12.6) (B, right) bound to RyR2 in the different experimental groups. In heart failure, RyR2 is significantly hyperphosphorylated by PKA (B, left), which leads to dissociation of calstabin2 (FKBP12.6) from the channel complex (B, right). Treatment with JTV-519 resulted in normalization of both PKA-phosphorylation status of RyR2, as well as FKBP12.6 binding to RyR2. The number of experiments is indicated in bars. *P<0.05, HF vs. sham; #P<0.05, HF+JTV-519 vs. HF

FIG. **6** illustrates that JTV-519 normalizes RyR2-channel gating in failing hearts. RyR2 channels were isolated from sham-operated (sham) or heart-failure (HF) rats. Representative single-channel tracings show that RyR2-channel open probability (Po) was significantly increased in failing hearts (middle), as compared with sham-operated rats (top). Treatment of heart-failure rats with JTV-519 for 4 weeks normalized channel open probability to levels similar to those of sham-operated animals. For each condition, the upper trace represents 5000 ms, and the bottom trace represents 200 ms. Channel openings are upward, the dash indicates the full level of channel opening (4 pA), and 'c' indicates the closed state of the channels. Amplitude histograms (right) revealed increased Po and subconductance openings in RyR2 channels from failing hearts.

FIG. **7** demonstrates that JTV-519 normalizes RyR2 channel gating by increasing FKBP12.6 (calstabin2) binding to RyR2 channels. (A) Wild-type RyR2 (RyR2-WT) channels were PKA phosphorylated in the absence or presence of the specific PKA inhibitor PKI_{5-24}, then incubated with calstabin2 (FKBP12.6) in the presence of JTV-519 at the indicated concentrations. The RyR2 immunoblot shows equal amounts of RyR2 in the samples; the calstabin2 immunoblot shows that JTV-519 enabled partial (100 nM) or complete (1000 nM) rebinding of calstabin2 to PKA-phosphorylated RyR2. (B) RyR2-S2809D, which mimics constitutively-PKA-phosphorylated RyR2, was incubated with calstabin2 in the presence of the indicated concentrations of JTV-519. The RyR2 immunoblot shows equal amounts of RyR2 in the samples; the calstabin2 immunoblot shows that JTV-519 enables partial (100 nM) or complete (1000 nM) rebinding of calstabin2 to RyR2-S2809D. (C) [^{35}S]-labeled calstabin2 binding curves show that JTV-519 increases binding affinity of calstabin2 to PKA-phosphorylated RyR2 and RyR2-S2809D mutant channels, to a level comparable to non-phosphorylated RyR2-WT. (D-F) Single-channel studies show that JTV-519 (1 μM) reduces the open probability (Po) of PKA-phosphorylated RyR2-WT by rebinding calstabin2 at 150 nM [Ca^{2+}] (n=11 for D; n=12 for E; n=13 for F). Channel openings are upward, the dash indicates the full level of channel opening (4 pA), and 'c' indicates the closed state of the channels. Amplitude histograms (right) reveal increased Po and subconductance openings in PKA-phosphorylated RyR2; this was not observed following treatment with JTV-519 (1 μM) and calstabin2 (FKBP12.6).

FIG. **8** illustrates the RyR2 macromolecular complex in atrial tissue. (A) RyR2 was immunoprecipitated from atrial sarcoplasmic reticulum (SR), and phosphorylated with PKA or cyclic adenosine monophosphate (cAMP). Addition of PKA inhibitor (PKI) completely blocked the phosphorylation reaction. (B) Components of the RyR2 macromolecular complex were co-immunoprecipitated with RyR2 from atrial SR. The positive control was atrial SR (with 50% of immunoprecipitation (IP) input). The negative control represents samples immunoprecipitated with antibody blocked with the antigenic peptide. (C) Calstabin2 (FKBP12.6) was co-immunoprecipitated with RyR2 from atrial SR. Prior to size-fractionation by SDS PAGE, samples were phosphorylated with PKA in the presence and absence of PKI. PKA phosphorylation caused dissociation of calstabin2 (FKBP12.6) from the channel complex, in a manner inhibited by PKI. +Cont. (CSR)= atrial SR; +Cont. (FKBP)=recombinant FKBP; -Cont. =IP performed with antibody pre-absorbed with antigenic peptide.

FIG. **9** shows PKA-hyperphosphorylation of RyR2 in atrial fibrillation (AF). (A) Immunoprecipitated (IP) RyR2 from control animals (Control; n=6) and dogs with atrial fibrillation (A Fib; n=6) was phosphorylated with PKA. For back-phosphorylation experiments, immunoblotting for RyR2 was performed in parallel, in order to determine the amount of RyR2 protein that was immunoprecipitated in each sample. The bar graph on the left represents a quantification of the back-phosphorylation studies. Values represent the relative degrees of PKA phosphorylation of RyR2, adjusted for the amount of immunoprecipitated protein. Dogs with AF showed a 130% increase in PKA phosphorylation, as compared with controls (n=6 for AF; n=6 for control; P=0.001). Calstabin2 (FKBP12.6) was co-immunoprecipitated with RyR2. For co-immunoprecipitation experiments, an immunoblot for RyR2 was performed in parallel, in order to determine the amount of RyR2 protein that was immunoprecipitated from each sample. The bar graph on the right represents a quantification of the co-immunoprecipitation experiments. Values represent the amount of calstabin2 (FKBP12.6) co-immunoprecipitated with RyR2, adjusted for the amount of immunoprecipitated protein. Calstabin2 (FKBP12.6) binding to RyR2 showed a 72% decrease in AF dogs, as compared with controls (n=6 for controls; n=7 for AF; P<0.0005). (B) An identical series of experiments was performed using human atrial tissue from patients with atrial fibrillation in the setting of heart failure (A Fib; n=5) and atrial tissue from patients with normal hearts (Control; n=3). The bar graph on the left represents a quantification of the back-phosphorylation studies. Humans with AF showed a 112% increase in PKA phosphorylation, as compared with controls (n=5 for A Fib; n=3 for Control; P=0.002). The bar graph on the right represents results of calstabin2 (FKBP12.6) co-immunoprecipitation experiments. Humans with AF showed a 70% decrease in the amount of calstabin2 (FKBP12.6) bound to RyR2 (n=5 for A Fib; n=3 for Controls; P<0.0001).

FIG. **15** demonstrates the experimental protocol used to test effects of the inventors' novel JTV-519-related compounds (disclosed herein) on hERG-channel current. Whole-cell patch-clamp experiments were carried out with physiological solutions at room temperature, in CHO cells transfected with hERG channel. Voltage-clamp protocols are shown in the lower panels. In vehicle, 0.1% DMSO in the external solution was applied with the same time-protocol as that shown in the upper panel.

FIG. **16** illustrates the effects of JTV-519 and the inventors' novel JTV-519-related compound, S36 (disclosed herein), on hERG-channel currents elicited by 80-mV depolarization. Representative hERG-channel currents (I(Kr)) were recorded from CHO cells before (open circle) and after (closed circle) application of 1 μM JTV-519 (left panel) or 1 μM JTV-S36 (right panel). The voltage-clamp protocol is shown below the current traces. Currents were elicited during 400-msec depolarization to +80 mV, from a holding potential of −90 mV. It should be noted that, upon the 400-msec depolarization (which mimics the human action potential duration (APD)), hERG channels pass very little outward current, because they rapidly inactivate. Tail currents marked by circles in current traces were elicited by return of the membrane potential to −40 mV, in the recovery from inactivation through the open state. Because the tail current is a major contributor to control of the APD, effects of the drugs were evaluated by tail currents at −40 mV: JTV-519=83% block; JTV-S36=39% block.

FIG. **17** shows effects of JTV-519, E4031, and the inventors' novel JTV-519-related compound, S36 (disclosed herein), on activation of hERG-channel currents (traces). Representative hERG-channel I-V relationships are shown before (control, left panels) and after (central panels) application of 0.1% DMSO (vehicle; upper central panel), 1 μM JTV-519 (middle central panel), and 1 μM JTV-S36 (lower central panel). The right panel shows that 5 μM E4031 (a class III anti-arrhythmic drug known to block hERG channels) completely blocked hERG-channel currents. (Note the tail currents at −40 mV). The voltage-clamp protocol is set forth in FIG. **15**, as an I-V relationship.

FIG. **18** demonstrates effects of JTV-519 and the inventors' novel JTV-519-related compound, S36 (disclosed herein), on activation of hERG-channel currents. The hERG-channel I-V relationships are shown for peak tail currents (activation) before (open squares) and after (closed squares) application of 0.1% DMSO (vehicle; upper panel), 1μM 15 JTV-519 (lower left panel), and 1μM JTV-S36 (lower right panel). Washout of the drugs is depicted with open triangles. The voltage-clamp protocol is set forth in FIG. **4**, as an I-V relationship. It should be noted that JTV-S36 did not block hERG currents at negative potentials (0 mV; 20 mV depolarization) showing voltage-dependent block of I(Kr).

FIG. **10** illustrates altered RyR2-channel function in AF. (A) Top traces show representative RyR2 channels from left atria of controls; lower traces are AF channels. To the right of the traces are the corresponding current amplitude histograms. (B) Bar graphs show quantification of open probability (Po) and frequency of opening (Fo) for control dogs (Cont.) and dogs with chronic atrial fibrillation (A Fib). 17 channels from 5 A Fib dogs, and 11 channels from 5 control dogs, were studied. Channels from the control dogs did not demonstrate increased activity. In contrast, 15 of 17 channels (88%) from A Fib dogs showed significantly increased open probability (AF: 0.39±0.07; control: 0.009±0.002; $P<0.001$) and gating frequency (AF: 21.9±4.6 s^{-1}; control: 1.6±0.6 s^{-1}; $P<0.002$).

FIG. **11** demonstrates that treatment with JTV-519 restores normal RyR2 function in AF. (A) Representative traces of single RyR2 channels from dog hearts, at a cytosolic Ca^{2+} concentration of 150 nM (as occurs during diastole) and in the presence of 0.25 mM calstabin2 (FKBP12.6), demonstrate significantly increased open probability (Po) and gating frequency after PKA phosphorylation (control: Po=0.3±0.2%, n=6; PKA: Po=14.8±3.2%, n=7; $P<0.001$). As seen at a higher time resolution in the lower trace, and in the all-point histogram, PKA phosphorylation of RyR2 results in partial openings (subconductance states) that are observed when calstabin2 (FKBP12.6) is dissociated from RyR2. JTV-519 (1.0 mM) restored channel activity of PKA-phosphorylated RyR2 (Po=0.8±0.3%; n=6; $P<0.001$), as compared with PKA-treated RyR2; JTV-519 also resulted in a discrete current amplitude distribution in the histogram, as seen in unphosphorylated control channels. The upper and lower traces represent 5000 msec and 200 msec, respectively; the closed state is indicated by 'c'; full channel openings are shown as upward deflections to 4 pA level, as indicated by the bars; the dotted lines in the lower traces indicate 1 pA steps of partial openings. (B) Recombinant calstabin2 (FKBP12.6) was incubated with PKA-phosphorylated RyR2, in the presence or absence of the 1,4-benzothiazepine derivative, JTV-519. Immunoblotting with anti-calstabin2 antibody revealed that JTV-519 allowed recombinant calstabin2 (FKBP12.6) to bind to PKA-phosphorylated RyR2. In the absence of JTV-519, calstabin2 binding did not occur.

FIG. **12** shows that novel 1,4-benzothiazepine derivatives induce binding of calstabin2 (FKBP12.6) to PKA-phosphorylated cardiac ryanodine receptor (RyR2) at 0.5 nM. The structures of the derivatives are set forth in Appendix A. upper panel=2.0 nM of each compounds; lower panel=0.5 nM of each compound

FIG. **13** shows that the 1,4-benzothiazepine derivative, S36 (depicted in Appendix A), prevents cardiac arrhythmias in mice at 200 nM. The bar graphs illustrate arrhythmic events or sudden cardiac death during exercise testing in $FKBP12.6^{+/-}$ mice—with or without drug treatment, as indicated. The left graph illustrates sudden cardiac death; the middle graph illustrates sustained VT; and the right graph illustrates non-sustained VT. Numbers refer to the total number of animals used in each group.

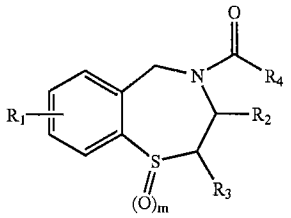
FIG. **14** demonstrates that JTV-519 improves cardiac contractility in a rat model of heart failure.

DETAILED DESCRIPTION OF THE INVENTION

Phosphorylation of cardiac RyR2 by protein kinase (PKA) is an important part of the "fight or flight" response; it increases cardiac EC-coupling gain by augmenting the amount of Ca^{2+} released for a given trigger (Marks, A. R., Cardiac intracellular calcium release channels: role in heart failure. *Circ. Res.*, 87:8-11, 2000). This signaling pathway provides a mechanism by which activation of the sympathetic nervous system, in response to stress, results in increased cardiac output required to meet the metabolic demands of the stress responses. Upon binding of catecholamines, β1- and β2-adrenergic receptors activate adenylyl cyclase via a stimulatory G-protein, $G\alpha_s$. Adenylyl cyclase increases intracellular cyclic adenosine monophosphate (cAMP) levels, which activate the cAMP-dependent PKA. PKA phosphorylation of RyR2 increases the open probability of the channel by dissociating calstabin2 (FKBP12.6) from the channel complex. This, in turn, increases the sensitivity of RyR2 to Ca^{2+}-dependent activation (Hain et al., Phosphorylation modulates the function of the calcium release channel of sarcoplasmic reticulum from cardiac muscle. *J. Biol. Chem.*, 270:2074-81, 1995; Valdivia et al., Rapid adaptation of cardiac ryanodine receptors: modulation by Mg^{2+} and phosphorylation. *Science*, 267:1997-2000, 1995; Marx et al., PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*, 101:365-76, 2000).

Failing hearts (e.g., in patients with heart failure and in animal models of heart failure) are characterized by a maladaptive response that includes chronic hyperadrenergic stimulation (Bristow et al., Decreased catecholamine sensi-

6. A method for reducing the risk of sudden cardiac death, sustained ventricular tachycardia and non-sustained ventricular tachycardia in a subject, comprising administering an effective amount of an agent to the subject, wherein the agent has the formula:



wherein

R_1=OR' at position 7 on the benzothiazepine ring;

R' =alkyl;

R_2=H;

R_3=H;

R_4=halide, carboxylic acid, or an alkyl-O— or —S-alkyl-S—; and

m=0, 1, or 2.

7. The method of claim 6, wherein the agent is administered to a subject that has or is at risk of developing a condition selected from the group consisting of cardiac arrhythmia, tachycardia, ventricular arrhythmia, ventricular fibrillation, ventricular tachycardia, sustained ventricular tachycardia, non-sustained ventricular tachycardia, catecholaminergic polymorphic ventricular tachycardia (CPVT), heart failure, sudden cardiac death and exercise-induced sudden cardiac death.

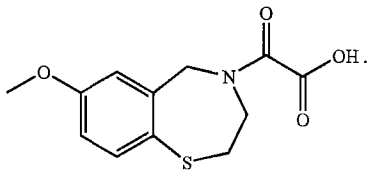
8. The method of claim 6, wherein the effective amount of the agent is one or more of:

(a) from about 5 mg/kg/day to about 20 mg/kg/day,

(b) an amount resulting in a plasma concentration of from about 0.02 μM to about 1.0 μM in the subject, or

(c) an amount resulting in a plasma concentration of from about 300 ng/ml to about 1000 ng/ml in the subject.

9. The method of claim 6, wherein the agent is



10. The method of claim 6, wherein the subject is a human.

11. The method of claim 1, wherein R_4=carboxylic acid and m=0 or 1.

12. The method of claim 1, wherein m=0 or 1.

13. The method of claim 12, wherein R_4=carboxylic acid and R'=methyl.

14. The method of claim 13, wherein m=0; and R_4=carboxylic acid.

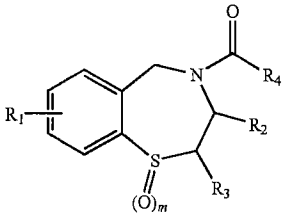
15. The method of claim 6, wherein R_4=carboxylic acid and m=0 or 1.

16. The method of claim 6, wherein m=0 or 1.

17. The method of claim 16, wherein R_4=carboxylic acid and R'=methyl.

18. The method of claim 17, wherein m=0; and R_4=carboxylic acid.

19. A method for treating cardiac arrhythmia in a subject, comprising administering an effective amount of an agent to the subject, wherein the agent has the formula:



wherein

R_1=OR' at position 7 on the benzothiazepine ring;

R' =alkyl;

R_2=H;

R_3=H;

R_4=halide, carboxylic acid, or an alkyl-O— or —S-alkyl-S—; and

m=0, 1, or 2.

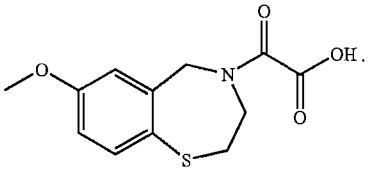
20. The method of claim 19, wherein the effective amount of the agent is one or more of:

(a) from about 5 mg/kg/day to about 20 mg/kg/day,

(b) an amount resulting in a plasma concentration of from about 0.02 μM to about 1 μM in the subject, or 1.0 μM

(c) an amount resulting in a plasma concentration of from about 300 ng/ml to about 1000 ng/ml in the subject.

21. The method of claim 19, wherein the agent is



22. The method of claim 19, wherein the subject is a human.

23. The method of claim 19, wherein R_4=carboxylic acid and m=0 or 1.

24. The method of claim 19, wherein m=0 or 1.

25. The method of claim 24, wherein R_4=carboxylic acid and R'=methyl.

26. The method of claim 25, wherein m=0; and R_4=carboxylic acid.

27. The method of claim 5, wherein the subject is a human.

28. The method of claim 5, wherein the subject has a cardiac condition selected from the group consisting of cardiac arrhythmia, tachycardia, ventricular arrhythmia, ventricular fibrillation, ventricular tachycardia, sustained ventricular tachycardia, non-sustained ventricular tachycardia, catecholaminergic polymorphic ventricular tachycardia (CPVT), heart failure, sudden cardiac death and exercise-induced sudden cardiac death.

29. The method of claim 5, wherein the effective amount of the agent is one or more of:

(a) from about 5 mg/kg/day to about 20 mg/kg/day,

(b) an amount resulting in a plasma concentration of from about 0.02 μM to about 1.0 μM in the subject, or

(c) an amount resulting in a plasma concentration of from about 300 ng/ml to about 1000 ng/ml in the subject.

* * * * *